

# Micro RNAs Versus Traditional Biomarkers in Predicting Reperfusion Injury in Hepatitis C Patients after Major Surgery

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## ABSTRACT

**Background:** Exposure to anesthesia in patients with chronic hepatitis C (CHC) may induce hepatic ischemia / reperfusion (I/R) with subsequent biochemical changes, provoked by alteration in molecular processes. This study explored miR-122 and miR-223 expression changes induced by anesthesia in CHC patients and their role in predicting hepatic I/R injury.

**Methods:** This study included two hundred ASA II - III adult patients, scheduled for major surgery under general anesthesia. Patients were divided into 2 main groups; CHC patients group and non-hepatitis patients group. Each group was further divided into 2 equal subgroups (50 patients each) according to type of anesthesia received either Sevoflurane SC /S or Propofol PC/P ). Blood samples were analyzed for liver enzymes, prothrombin time, and serum lactate at several intervals; preoperative, 12 and 48 hours postoperative. Also, plasma miR-122 and miR-223 were estimated by Reverse Transcription PCR preoperative and 12 hours postoperative.

**Results:** CHC groups showed significantly increased preoperative expression of both miR-122 and miR-223 compared to non-hepatitis groups (P<0.05). Moreover, postoperative samples revealed more exaggerated expression miR-122 and miR-223. Receiver operating characteristic curve (ROC) results revealed; values for area under the ROC for miR-122, miR-223, ALT and serum Lactate were 0.991, 0.965, 0.746 and 0.732 respectively. Based on the ROC finding, for prediction IR in CHC patients receiving anesthesia miR-122 had best Accuracy, sensitivity, and specificity.

**Conclusion:** Our results suggested that both miR-122 and miR-223 could be regarded as more sensitive and specific biomarkers for predicting hepatic I/R injury than traditional biomarkers particularly in CHC patients undergoing major surgery.

**Keywords:** miRNA; Chronic Hepatitis C; Ischemia; Reperfusion; Propofol; Sevoflurane

## Introduction

Patients with Chronic Hepatitis C (CHC) are at predominantly high risk for morbidity and mortality as result of the effects of general anesthesia and stress of surgery. [1] Since, the reciprocal flow relation between the hepatic artery and the portal vein is not well maintained, liver architecture is disrupted by fibrosis with consequent alteration of the normal patterns of liver blood flow which makes such patients more prone to ischemia/reperfusion (I/R). [1,2]

Ischemia reperfusion (I/R) is defined as diminished oxygen supply to tissues to concentrations that are insufficient to maintain physiologic cell function. Reperfusion is essential for survival of ischemic tissue. [3] However, reperfusion can injure ischemic tissue when the blood supply returns to the tissue after a period of ischemia. Since, absence of oxygen from blood during the ischemic period creates a condition in which the restoration of circulation results in inflammation and oxidative damage through release of cytokines from activated endothelium and tissue macrophages, endothelial adhesion/ dysfunction and migration of neutrophils. [3,4]

Exposure to anesthesia in CHC patients may aggravate hepatic ischemia/reperfusion(I/R) with subsequent physiological and biochemical outcomes. These outcomes are somewhat mediated by alteration in molecular processes such as transcription and translation. The gold standard method to evaluate degree of liver damage after I/R is by measuring serum aspartate aminotransferase (AST) and alanine . aminotransferase (ALT) levels and lactate dehydrogenase (LDH). However, interpretation of these enzymes levels may be difficult due to their existence in other tissues. [5] MicroRNAs (miRNAs) are small non-coding RNAs that are implicated in regulation of messenger RNA degradation or translational repression. miRNAs negatively regulate more than 30% of the genes in cells, and control cell proliferation, inflammation, and metabolism. [6] Moreover, miRNAs are extremely stable and are resistant to degradation; Since, plasma miRNAs are bound to high-density lipoprotein and to Argonaute-proteins, which stabilizes them for long periods. [7] A number of specific miRNAs are modulated in liver diseases; particularly, miR-122 is expressed in solely in hepatocytes, and hepatic

cell death and necro-inflammatory activity in patients with CHC results in elevated expression of plasma miR-122. Also, experimental studies revealed change in expression of plasma miR-223 in I/R. [8] However, the role of miR-223 in I/R injury in humans has seldom been reported. [6]

Sevoflurane and propofol anesthesia are widely used for anesthesia and may influence hepatic blood flow, oxygen consumption and hepatic functions in the perioperative period, and they may influence the expression pattern of miRNA in CHC patients undergoing major surgery. [1]

This study investigated miR-122 and miR-223 expression changes induced by sevoflurane and propofol anesthesia in CHC patients and the role of these novel biomarkers in predicting hepatic injury during anesthesia versus traditional biomarkers.

## Patients and Methods

The study was carried out in Alexandria Main University Hospital between January 2016 and January 2017. It was designed as double blind study, patients were assigned to groups at random. The study included 200 adult patients, ASA physical status II - III, scheduled for major surgeries with expected excessive blood loss ( orthopedic surgery; spinal fixation, pelvic or compound femur fractures) under general anesthesia (GA). The research protocol was approved by the Ethics committee of the Faculty of Medicine, Alexandria University, Egypt. As well as in accordance with the ethical standards laid down in the 1964 declaration of Helsinki. Informed written consent was obtained from all participating patients.

**Patients:** All patients were subjected to full medical history, thorough clinical examination, laboratory investigation and ultrasound examination. They were divided into four groups; two groups were CHC patients and two groups were non-hepatitis patients. CHC patients were diagnosed by assays for HCV antibody testing, by using of a commercial recombinant immunoblot assay (Innolia HCV Ab III; Innogenetics) and confirmed by real-time quantitative HCV RNA PCR (more than 50 IU/ml).

The Child-Pugh scoring system was performed for all CHC patients to assess the severity of cirrhosis, using grades for the following parameters of bilirubin, albumin, prothrombin time, hepatic encephalopathy and ascites from one to three to a maximum score of 15. [9] Non of patients in this study had Child-Pugh Class C cirrhosis. Exclusion criteria included pregnancy, malignancy, pulmonary or cardiovascular disease, concomitant Hepatitis B infection.

Studied patients were divided into two main groups ; CHC group and non-hepatitis group (one hundred patients each). Chronic hepatitis C (CHC) group (one hundred) were further

divided into 2 subgroups according to anesthetic agent used; SC group (CHC patients who received Sevoflurane anesthesia ) and PC group (CHC patients who received propofol anesthesia ) , while non- hepatitis patients (one hundred) were divided into 2 subgroups: S group ( non-hepatitis patients who received Sevoflurane anesthesia ) and P group (non-hepatitis patients who received propofol anesthesia ). On arrival to operation room, patients were connected to (Trakmon-Kontron-Limited- England) multichannel monitor for routine monitoring and two large (16 gauge) intravenous cannulas were inserted.

**Laboratory Investigations:** Blood samples were collected pre-operative (before induction of anesthesia), 12 hours and 48 hours post-operative to determine the following tests: Serum samples for liver enzymes; serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT), Blood samples were collected into Vacutainer tubes containing 0.129 M sodium citrate: prothrombin time (PT) and partial thromboplastin time (PTT) measured by a STA analyzer (DiagnosticaStago, Asnieres-sur-Seine, France), and EDTA blood for complete blood picture (CBC), determined by automated cell counter (ERMA INC-Tokyo. Model PCE-210). Also serum lactate levels were measured within 15-30 minutes using a point-of-care device, samples were kept on ice to avoid falsely elevated levels of lactate. In addition, blood samples were collected in EDTA tubes, centrifuged at 4000 rpm for 10 min at 4 °C, plasma was separated and stored at -70°C for analysis of miR-122 and miR-223, samples were withdrawn pre-operatively and 12 hours post-operatively.

## miRNA Quantification by Real-Time PCR (RT-qPCR)

**RNA extraction and cDNA synthesis:** Total RNA was extracted from 200 µl plasma using the miRNeasy extraction kit (Qiagen, Valencia, CA, USA). RNA purity was assessed by the RNA concentration and quantified by NanoDrop Spectrophotometer (Nanodrop ND-1000, United States). Followed by analysis using agarose gel electrophoresis. Reverse transcription was performed for cDNA synthesis using the miRNeasy plasma Reverse Transcription Kit (Qiagen, Valencia, CA, USA) according to the manufacturer's instructions. Amplification and quantification

The expression miR-122 and miR-223 were evaluated by RT-qPCR analysis according to the manufacturer's protocol. The housekeeping miRNA SNORD 68 was used as the endogenous control. For real-time PCR, the cDNA template was mixed with SYBER Green Master Mix (Qiagen, Valencia, CA, USA). PCR array plate enriched with forward and reverse miRNA-specific primers.

Real-time PCR reactions were performed using an Applied Biosystems 7500 Real Time PCR System (Foster city, CA, USA) with the following conditions: 95°C for 15 min, followed by 40 cycles at 94°C for 15 s, 55°C for 30 s and 70°C for 34 s. The cycle threshold (CT) is defined as the number of cycles required for the fluorescent signal to cross the threshold in real-time PCR.

Expression of miRNAs was reported as  $\Delta$ Ct value. The  $\Delta$ Ct was calculated by subtracting the Ct values of miRNA SNORD68 from the Ct values of the target miRNAs. there is an inverse correlation between  $\Delta$ Ct and miRNA expression level, lower the  $\Delta$ Ct values were associated with increased miRNA. The resultant normalized  $\Delta$ Ct values were used in calculating relative expression values by using  $2^{-\Delta(\text{Ct})}$ , these values are directly related to the miRNA expression levels. The  $2^{-\Delta\Delta(\text{Ct})}$  method was used to determine relative-quantitative levels of individual miRNAs.

**Anesthetic Protocol:** All patients were subjected to the same anesthetic protocol for induction of GA, using intravenous (IV) propofol 2-3 mg/kg and IV fentanyl 2  $\mu$ g/kg; endotracheal intubation was facilitated by IV atracurium 0.5 mg/kg. Anesthesia in Groups S and SC patients were maintained by 2-3% sevoflurane in oxygen and atracurium increments, while anesthesia in Groups P and PC patients were maintained by propofol infusion at a rate of 6 mg/kg/hr and IV atracurium 0.5 mg/kg increments. In addition to standard monitoring [Heart rate (HR), Mean arterial blood pressure (MAP) and Pulse oxygen saturation ( $\text{SpO}_2$ )] urinary output was recorded. At the end of surgery, residual neuromuscular block was antagonized with IV neostigmine 0.05 mg/kg and atropine 0.02 mg/kg.

Fluid therapy was managed by administration of crystalloids and colloids solutions. Adequate replacement and maintenance of intravascular volume were guided by monitoring MAP, urinary output ( $\geq 1$  ml /kg/h) and hourly hematocrit measurement. Packed cells (PEs) was transfused when the hemoglobin decreased to less than 10 g/dl, to prevent inadequate oxygenation during rapid bleeding. Other blood products, such as fresh frozen plasma (FFP) and platelets were administered intraoperative in accordance with the recommendations of the ASA Task Force on Blood Component Therapy. [10] Operative time was recorded in minutes. Estimated blood volume loss during surgery was determined from the surgical suction reservoir and by weighing towels from the operative field; total intraoperative blood transfusion was calculated.

**Statistical Analysis:** The raw data were coded, entered, and analyzed using IBM SPSS Software Package version 20, The 0.05 level was used as the cut off value

for statistical significance and the following statistical measures were used: Descriptive statistics including counts and percentages, Arithmetic mean, standard deviation, for categorized parameters, chi square test was used while for numerical data t-test was used to compare two groups while for more than two groups ANOVA test was used, followed by post hoc test to determine the level of significant between each two groups, it was done Duncan method, by using the small letters, the same letters indicate that there was no significant difference, while the different small letters show a significant difference. To find the association between two variables, Spearman correlation coefficient test was used, the level of significant was 0.05.

## Results

This study included two hundred adult patients who underwent major orthopedic surgery with blood loss at Alexandria University hospital during study period. Patients were randomly divided into four equal groups (50 each); 2 CHC groups (SC and PC), and 2 non-hepatitis groups (S and P). CHC patients included in study were classified according to Child-Pugh scoring; SC group (CHC patients who received Sevoflurane) 14 patients were class A and 36 patients were class B, while PC group (CHC patients who received propofol) 10 patients were class A and 40 patients were class B.

No significant differences were detected as regards age, gender, surgical time, urine output, blood loss and body temperature. None of the non-hepatitis groups (S and P) received blood transfusion, while 7 patients in SC group received 2 units of packed red blood cells each, while 2 patients in PC group received 2 units of packed red blood cells each. (Table 1)

MAP measures were significantly lower in both groups that received sevoflurane anesthesia (SC and S) than groups that received propofol anesthesia (PC and P) at the end of surgery, with a more significant decrease in SC group compared to other groups. (Table 2) Platelets count was significantly lower in CHC groups (SC and PC) than non-hepatitis groups (S and P); pre-operative, 12 hrs and 48 hrs post-operative. (Table 3)

PT values were significantly prolonged in both hepatitis groups (SC and PC) compared to non-hepatitis groups (S and P); Preoperative ( $p_1=0.012$  and  $p_2=0.011$ ), 12 hours post-operative ( $p_1=0.033$  and  $p_2=0.021$ ) and 48 hours post-operative ( $p_1=0.003$  and  $p_2=0.015$ ). with a significantly higher % change in CHC group SC group compared to PC group ( $P \leq 0.05$ ). Also, Serum AST levels were significantly elevated in hepatitis groups (SC and PC) compared to non-hepatitis groups (S and P); Preoperative ( $p_1=0.002$  and  $p_2=0.001$ ), 12 hours post-operative ( $p_1=$

0.001 and  $p_2=0.001$ ) and 48 hours post-operative ( $p_1=0.001$  and  $p_2=0.001$ ) with a significantly higher % change in CHC group SC compared to PC group ( $P \leq 0.05$ ). (Table 3)

Preoperative plasma expression miR-122 and miR-223 were significantly increased in both CHC groups (SC and PC) compared to non-hepatitis groups (S and P). Post-operative samples (after 12 hours) plasma miR-122 showed significantly increased expression in both CHC groups compared to non-hepatitis groups. Moreover, SC group revealed more significant increased expression compared to other groups. Similarly postoperative plasma miR-223 revealed more significant expression non-hepatitis group S (receiving Sevoflurane) relative group P (receiving Propofol). Also postoperative analysis revealed more significant increase in CHC groups with

more pronounced expression in SC group compared to PC group. (Table 4)

Analysis of plasma miR-122 displayed a significant increase in CHC patients SC group (receiving Sevoflurane) with child pugh B both preoperative ( $P : 0.042$ ) and 12 hours postoperative ( $P: 0.024$ ). Also, plasma miR-223 was significantly increased in patients with child pugh B both preoperative ( $P: 0.011$ ) and 12 hours postoperative ( $P: 0.016$ ) in CHC patients in SC group compared to PC group. In the PC group miR-122 preoperative ( $P : 0.069$ ) and 12 hours postoperative was ( $P: 0.077$ ); while miR-223 preoperative ( $P : 0.032$ ) and 12 hours postoperative was ( $P: 0.036$ ).

ROC curve was designed for predicting hepatic IR in CHC patients receiving anesthesia, results revealed that miR-122 had 97% sensitivity, 92% specificity and 95% accuracy (Figure1, Table 5)

**Table 1: Demographic & Physiological Data for all studied groups.**

	SC	S	PC	P
Age	47.09±6.23	44.45±7.78	49.67±5.56	46.23±7.10
P1	0.213		0.336	
P2			0.112	0.207
Gender (M/F)	(12/8)	(10/10)	(12/8)	(11/9)
P1	0.425		0.365	
P2			1.00	0.854
Duration of operation(min)	139±18	132±13	146±19	139±21
P1	0.236		0.411	
P2			0.078	0.365
Blood loss(ml)	1078±271	916±129	1012±254	930±168
P1	0.107		0.116	
P2			0.689	0.722
Volume of fluid infused(ml)	1800±420	1680±510	2000±495	1780±470
P1	0.031*		0.041*	
P2			0.101	0.223
Urine output	355±165	368±159	386±145	377±148
P1	0.652		0.482	
P2			0.136	0.254
Temperature at the end of surgery (°C)	36.0±0.4	36.5±0.4	35.9±0.3	36.7±0.5
P1	0.682		0.211	
P2			0.365	0.852

\* Statistically significant at ( $P \leq 0.05$ )

SC: CHC patients who received Sevoflurane, S: non-hepatitis patients who received Sevoflurane, PC: CHC patients who received Propofol, P: non-hepatitis patients who received Propofol.

P1: between groups receiving same anesthetic drug.

P2 : between 2 control groups and 2 hepatitis groups.

**Table 2: Hemodynamic measurements for all studied groups during surgery.**

	SC	S	PC	P
<b>Mean arterial blood pressure (mmHg):</b>				
*Preoperative	94±21	93±17	103±15	98±20
P1	0.311		0.207	
P2			0.032*	0.041*
*Intra-operative (30 min after start of surgery)	88±14	90±13	102±17	96±18
P1	0.103		0.412	
P2			0.001*	0.046*
*At the end of surgery	84±20	90±18	104±17	94±16
P1	0.041*		0.106	
P2			0.002*	0.107
<b>Heart rate (beat per min):</b>				
* Preoperative	75±15	78±14	67±12	73±14
P1	0.365		0.085	
P2			0.017*	0.165
*Intra-operative (30 min after start of surgery)	72±10	77±15	70±13	75±12
P1	0.071		0.103	
P2			0.235	0.365
*At the end of surgery	71±14	74±16	67±17	73±15
P1	0.336		0.144	
P2			0.185	0.236

\* Statistically significant at ( $P \leq 0.05$ )

SC: CHC patients who received Sevoflurane, S: non-hepatitis patients who received Sevoflurane. While PC: CHC patients who received Propofol, P: non-hepatitis patients who received Propofol.

P1: between groups receiving same anesthetic drug.

P2 : between 2 control groups and 2 hepatitis groups.

**Table 3: laboratory investigations measured preoperative, postoperative after 12 hours and 48 hours in all studied groups.**

	SC	S	PC	P	P 1	P 2	P 3
<b>ALT (IU/ml):</b>							
*Preoperative	47.43±12.45	28.67±14.23	62.87±15.88	23.45±11.89	0.022*	0.001*	0.016*
*12 hrs post-op.	110.68±41.77	44.23±19.65	112.78±72.69	45.43±13.89	0.003*	0.001*	0.211
*48 hrs post-op.	398.32±56.56	36.41±17.13	291.37±61.38	38.26±18.67	0.001*	0.001*	0.017*
<b>Hemoglobin (gm/dl):</b>							
*Preoperative	11.6±2.8	14.1±1.6	11.3±2.1	14.1±0.7	0.039*	0.041*	0.699
*12 hrs post-op.	10.2±1.6	13.9±1.4	11.0±0.8	13.9±1.8	0.036*	0.068	0.356
*48 hrs post-op.	10.1±1.8	13.9±1.56	10.9±0.9	14.0±0.92	0.035*	0.041*	0.32
<b>Serum lactate (mmol/L)</b>							
*Preoperative	1.96±0.48	1.21±0.34	1.82±0.43	1.01±0.31	0.033*	0.041*	0.365
*12 hrs post-op.	5.67±1.98	2.66±1.78	4.54±1.88	2.83±1.91	0.003*	0.0021*	0.106
*48 hrs post-op.	3.23±1.77	1.85±1.04	2.25±1.52	1.71±1.1	0.026*	0.041*	0.107

\* Statistically significant at ( $P \leq 0.05$ )

SC: CHC patients who received Sevoflurane, S: non-hepatitis patients who received Sevoflurane, PC: CHC patients who received Propofol, P: non-hepatitis patients who received Propofol.

P1 comparison between S and SC.

P2 comparison between P and PC.

P3 comparison between SC and PC.

**Table 4: Plasma miR-122 and miR-223 expression preoperative and 12 hours postoperative in all studied groups.**

Plasma miR-122 in all studied groups							
Sevoflurane Anesthesia Groups			P1	Propofol Anesthesia Groups			P1
	Preoperative	12 hrs Postoperative			Preoperative	12 hrs Postoperative	
S	0.78±0.31	0.99±0.18	0.107	P	0.72±0.27	0.96±0.23	0.211
SC	7.02±1.77	8.92±1.07	0.203	PC	6.52±0.92	7.46±1.03	0.071
P2	0.001*	0.001*			0.001*	0.001*	
plasma miR-223 in all studied groups							
Sevoflurane Anesthesia Groups			P1	Propofol Anesthesia Groups			P1
	Preoperative	12 hrs Postoperative			Preoperative	12 hrs Postoperative	
S	0.811±0.041	1.02±0.23	0.032*	P	0.726±0.09	0.83±0.03	0.425
SC	4.21±0.33	6.07±1.07	0.013*	PC	3.96±0.285	5.33±0.18	0.021*
P2	0.001*	0.001*			0.001*	0.001*	

\* Statistically significant at ( $P \leq 0.05$ )

SC: CHC patients who received Sevoflurane, S: non-hepatitis patients who received Sevoflurane, PC: CHC patients who received Propofol, P: non-hepatitis patients who received Propofol.

P1 comparison between pre and post in the same group

P2 comparison between the two groups at the same time

**Table 5: Cut off value of different biomarkers evaluated to predict the IR injury in CHC patients undergoing major surgery.**

Test Result Variable(s)	Area under the curve	p-value	Cut off value	Sensitivity	Specificity	Accuracy	Asymptotic 95% Confidence Interval	
							Lower Bound	Upper Bound
ALT	.746	.012	32.0	75.0	72.0	74.0	.676	.815
Serum lactate	.732	.011	1.42	70.0	74.0	72.0	.661	.803
miR-122	.991	.0001	0.68	97.0	92.0	95.0	.976	1.000
miR-223	.965	.0001	0.75	95.0	90.0	92.0	.944	.987

**Table 5: Cut off value of different biomarkers evaluated to predict the IR injury in CHC patients undergoing major surgery.**

Test Result Variable(s)	Area under the curve	p-value	Cut off value	Sensitivity	Specificity	Accuracy	Asymptotic 95% Confidence Interval	
							Lower Bound	Upper Bound
ALT	.746	.012	32.0	75.0	72.0	74.0	.676	.815
Serum lactate	.732	.011	1.42	70.0	74.0	72.0	.661	.803
miR-122	.991	.0001	0.68	97.0	92.0	95.0	.976	1.000
miR-223	.965	.0001	0.75	95.0	90.0	92.0	.944	.987

## Discussion

Abundant evidence revealed the involvement of miRNAs in different forms of liver disease; detailed mechanisms of the influence of anesthetic agents on the liver miRNAs expression during I/R are still unknown. the two widely used general anesthetics propofol and sevoflurane, are

known to influence hepatic cell function during the perioperative period. [13] In the present study, it was investigated whether plasma miR-122 and miR-223 could serve as potential indicators of IR injury in CHC patients. Therefore, plasma expression levels of these miRNAs both preoperative and postoperative in patients undergoing

major surgery with expected I/R and were tested to determine whether their expression levels correlated with established enzyme of hepatocellular injury AST and ALT, as well as serum lactate levels.

Results of the current study showed elevated expression of plasma miR-122 in CHC groups (SC and PC) compared to non-hepatitis groups (S and P) in preoperative period. This was explained by the fact that miR-122 constitutes more than 70% of the total liver miRNA, and are released into circulation as result of hepatocyte injury due to HCV infection. Also, more exaggerated expression of plasma miR-122 in CHC groups in the postoperative period compared to non-hepatitis groups, with more prominently increased expression in CHC group who received Sevoflurane (SC). Exaggerated expression of plasma miR-122 was explained by further release into the blood stream as a result of hepatic cell insult due to hypoperfusion, inflammatory condition and/or drug induced liver injury. [14,15] In addition, plasma miR-122 levels were found to be significantly associated with liver function parameters; ALT, PT and serum lactate particularly in CHC groups. Similarly, other studies reported that circulating level of miR-122 correlates with severity of hepatocyte injury. [16,17]

Hepatic injury as result of I/R ensue from sinusoidal damage with subsequent microcirculatory disturbance upon reperfusion, resulting from prolonged hypotension and increased blood loss following hepatocyte injury. [18] Those outcomes were demonstrated by evaluation of liver function tests, serum lactate which reflect hepatocyte integrity. [15]

In present study liver enzymes; serum AST or ALT levels did not change significantly in 12 hours and 48 hours postoperative in both non-hepatitis groups (S and P). However, significantly elevated liver enzyme ALT and prolonged PT were observed in samples collected 12 and 48 hours post-operative in CHC groups with more significant increase in Sevoflurane group (SC). Those results determined presence of hepatic dysfunction in CHC patients with anesthesia particularly Sevoflurane, which was in agreement with results of Subhi et al study. [18] In addition, Choi et al reported that miR-122 regulates the levels of ALT without changing the transcriptional level of the coding gene, proposing that it is implicated in translation activation of ALT. [19]

Likewise, other studies confirmed that circulating miR-122 is proposed an early disease severity-dependent biomarker of liver injury as its levels become raised earlier than that of serum ALT. [18,19,20] Moreover, significant increase in liver enzymes in postoperative periods (after 12 and 48 hours) in CHC groups. Hepatic enzyme ALT is involved

in cellular nitrogen metabolism and gluconeogenesis by catalysing the transamination between alanine and 2-oxoglutarate, the preferential localisation of miR-122 around lipid droplets of the hepatocytes reinforces this perception. [21,22] Also, a novel role of miR-122 in the modulation of hepatic glucose metabolism and elucidated its profound relation with ALT. [20]

In addition, other studies stated that plasma miR-122 can be detected before histopathologic changes evolve in *experimental* models of cytotoxic liver-induced damage, and they agreed to consider miR-122 not only as a biomarker of hepatocellular necrosis, but also hepatocellular stress. [6,20,23,24]

Furthermore, sevoflurane is known to produce extra metabolite, Compound A [fluoromethyl-2,2-difluoro-1-(difluoromethyl) ether], through chemical reactions with CO<sub>2</sub> absorbents. [18] The resultant Compound A might be hepatotoxic as proved by transient increase in postoperative liver function tests as well as elevated plasma miR-122, miR-223 expression in our study. Also, a study performed on morbidly obese subjects undergoing surgery, reported that ALT has less specificity than miR-122 for identifying liver cell injury because of its presence in the heart and skeletal muscle. [17]

Moreover, significantly elevated serum lactate in CHC groups after 12 and 48 hours post-operative was the result of impaired lactate clearance due cellular hypoperfusion or hypoxia and anaerobic metabolism. [25]

As regard plasma miR-223; it functions as an important modulator of cellular differentiation, including dendritic cells and macrophage activation. [26] Furthermore, miR-223 influences the inflammatory response through activation of neutrophils and the secretion of inflammatory cytokines. [27] Furthermore, miR-223 in the liver imparts a potential role in modulation of the pharmacokinetics. This regulatory mechanism might be a supplementary factor for the inter- and intra-individual variability in drugs pharmacokinetics. Cytochrome b5 (b5) is post-transcriptionally regulated through miR-223. The b5 is a hemoprotein that transfers electrons to several enzymes to accomplish fatty acid desaturation, steroidogenesis, and drug metabolism. [28]

Hepatocyte damage in I/R is partially due oxygen-derived free radicals due to lipid peroxidation, especially during reperfusion. cytochrome b5 increases during ischemic periods, followed by immense increase 60 min after reperfusion. it exerts a protective effect against liver cell damage, such as free radical-trapping ability and or membrane stabilizing action. [28,29]

Pronounced expression of postoperative plasma miR-223 in CHC group (SC); could be explained by presence of additional mechanism of I/R includes damage caused by sustained accumulation of leukocytes; with consequent obstruction of microcirculation, release of non-enzymatic components for a prolonged period after their activation; furthermore produced cytokines might result in hepatocyte injury. [30,31] miR-223 might be useful as an indicator of liver I/R injury as its expression is elevated and was positively correlated with ALT level and elevated serum lactate 12 hours postoperative in both CHC groups (SC and PC). Accordingly, Yu et al suggested that miR-223 is a more sensitive indicator of hepatic I/R injury than traditional liver function tests (ALT and AST).[26]

Moreover, previous studies confirmed that propofol has a hepatoprotective effect through its role in reduction of free radical generation and free radical scavenging, dumping of inflammatory reactions. [32,33] It has inhibitory effects on calcium channels and diminution of intracellular calcium overload, improved microcirculation through reported induced nitric oxide (NO) and vasodilatory prostanoid production. [33,34]

ROC curves revealed that miR-122 had the highest efficiency in prediction of ischemia/reperfusion injury followed by miR-223. These findings suggested that estimation of plasma miR-122 and miR-223 in CHC undertaking anesthesia could be an early, and reliable biomarker for predicting I/R liver injury. Interestingly, these biomarkers were significantly related with CHC patients (SC and PC groups) with child-pugh score child-pugh B than A; thus these miRNAs could be considered as sensitive biomarkers for predicting liver stress and ischemia specifically in CHC patients receiving anesthesia.

Also, Sevoflurane is associated with increased risk of bleeding in I/R. As Sevoflurane produces prolonged bleeding times in normal patients, accordingly this effect is more exaggerated in CHC patients. The underlying mechanisms is thought to be through immediate inhibition of granulocyte-platelet aggregation by suppression of thromboxane A2 formation, inhibition of calcium mobilization in platelets and inhibition of platelet aggregation. [37,38,39]

Significantly reduced platelet count detected in our study predominantly in CHC groups. Concurring with other studies which reported reduced platelet counts, due to platelet accumulation in liver during I/R. [39,40,41] Kurokawa et al stated that platelets adhered to Kupffer cells at the early period of I/R and that those platelets were involved in apoptosis of hepatocytes and subsequently stimulated platelet migration from sinusoidal space into

the space of Disse. [42]

Significant reduction in MAP was observed in Sevoflurane anesthesia groups (S and SC) intra-operative and at the end of surgery may be the result of impaired auto-regulation. On the other hand, a more preserved hepatic function was perceived in groups receiving Propofol anesthesia (P and PC). Although it is known that CHC patients might have alterations in the systemic circulation due to arteriovenous shunting and reduced splanchnic inflow, which makes liver more vulnerable to hypoxemia and hypotension during anesthesia. [43,44]

## Conclusion

Our results suggested that both miR-122 and miR-223 could be considered more sensitive and specific predictors of hepatic I/R injury than the traditional parameters of liver function (as ALT and AST) and serum lactate in CHC patients. Additionally, both sevoflurane and propofol anesthesia affect miRNA expression. However, further studies are required to determine the functional consequences of miRNA modulation in I/R in CHC versus non-hepatitis patients.

## Declaration

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